THE INFLUENCE OF NUCLEIC ACID ON DEHYDROGENASE SYSTEMS A CONTRIBUTION TO THE PROBLEM OF GENE MECHANISM

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INTRODUCTION

The cellular components comprise a vast number of different compounds, salts, proteins, carbohydrates, fats, nucleates, etc., each in a state of metabolic flux, and each interacting with others to provide the energy for specific function, the supply of structural materials, and the disposal of breakdown products. This complicated and highly diverse mechanism of the cell is regulated by a mosaic of enzyme systems which are not independent but interrelated, and which are themselves regulated and affected by extrinsic and intrinsic factors and conditions. Thus, it is possible to affect the level of particular enzymes by nutritive deprivation of the cell, by the application of chemical inhibitors or stimulators, and by cellular mutations whether spontaneous or induced. Any attempt to elucidate these phenomena *in toto* is quite obviously a formidable task. The biochemist at present can study only a few systems at a time, under conditions often considerably removed from the physiological, and, as yet, with tools and concepts alike available only for first approximations. Biological chemistry owes its beginnings to the insight of men like Miescher, Kossel, and others, who believed that problems in tissue function might be at least partially explicable in terms of the chemical properties of isolated components and systems. It is with this hope undimmed, and with due recognition of the limitations inherent in the approach, that an attempt at a study of a chemical basis of gene action may be begun (Greenstein and Chalkley, '45).

Nucleic acid combined with specific proteins appears to yield conjugated compounds often with remarkable biological properties. Among these compounds are the viruses and the components of the chromosomes. Considerable information exists suggestive of a linking of nucleic acids with gene mechanisms, but in the absence of evidence for the isolated gene any attempt to identify the latter as a nucleoprotein must be treated with some reserve. Nevertheless, there is evidence that growth and reduplication are associated with the presence of nucleic acid, and the relation of the latter type of substance with the gene may thus be strongly inferred. Since the genic material exerts a controlling influence over the multitudinous functional processes of the cell, it may be assumed that it accomplishes this not by a remote form of control but by specific kinds of chemical interaction with the cellular components involved in individual reactions, i. e., enzymes (cf. Tatum and Beadle, '45). It is for this reason that the study of the chemical interaction of nucleic acid with various kinds of cellular components should yield sig-

nificant implications (Miescher, '97, Hammarsten and Hammarsten, '28, Greenstein and Jenrette, '41).

In the form of their neutral sodium salts, the nucleic acids in aqueous solution are highly elongated, polymerized molecules (cf. Greenstein, '44). The degree of asymmetry and the extent of polymerization of the desoxyribose nucleate (chromosomal component) are markedly decreased in the presence of proteins and of salts, and the relative amount of this decrease is a function of the nature, the state, and the concentration of the protein and of the salt. The molecular configuration of the nucleates is in particular highly labile toward the proteins.[1]

The question that naturally arises is whether there is some reciprocal effect of the nucleate on the molecular configuration of the proteins with which it interacts. This question is most readily answered by employing proteins with specific and readily measured properties, namely enzymes. Changes in the physical or chemical properties of these substances, due to the interaction with the nucleate, might be expected to be reflected in observable changes in their catalytic capacities. Furthermore, if such changes occurred, they would provide a possible clue to some of the mechanisms which chromosomal components exert in the maintenance and regulation of cellular functions.

PROCEDURE AND RESULTS

Aqueous tissue extracts containing reducing systems possess the capacity of decolorizing methylene blue under anaerobic conditions. We have observed that when sodium yeast nucleate (ribose nucleate) is added to such extracts, the decolorization rate is slightly decreased; when sodium thymus nucleate (desoxyribose nucleate) is added, this rate is very considerably decreased. The extent of this decrease in rate is proportional to the amount of nucleate added. Addition of xanthine results in an increase in decolorization rate (measure of xanthine dehydrogenase activity) which, at high levels of dye concentration, appears to be very nearly the same whether nucleate is present or not. The percentage increase in rate on addition of substrate, however, is greatest in the presence of thymus nucleate. Nearly identical results are obtained with freshly-mixed solutions and with mixtures which are allowed to stand until the viscosity of the thymus nucleate is reduced nearly to that of the extract (enzymatic depolymerization (Greenstein, '44)). The fact that the activity of xanthine dehydrogenase is independent of the presence of nucleate (at high dye concentrations) indicates that there is no effect of the nucleate on the dye. The results of a typical experiment are given in Table I (Greenstein and Chalkley, '45).

When the mixtures described in Table I are treated with smaller and smaller quantities of methylene blue, keeping the total volume constant by addition of water, data indicated by the curves in fig. 1 are obtained.

The exponential character of the curves in fig. 1 led us to replot the data given

[1] This configuration is also labile toward the effects of ultra-violet radiation (Hollaender, Greenstein, and Jenrette, '41), and this phenomenon is suggestive in connection with mutations induced by this agent (cf. Carlson and Hollaender, '44).

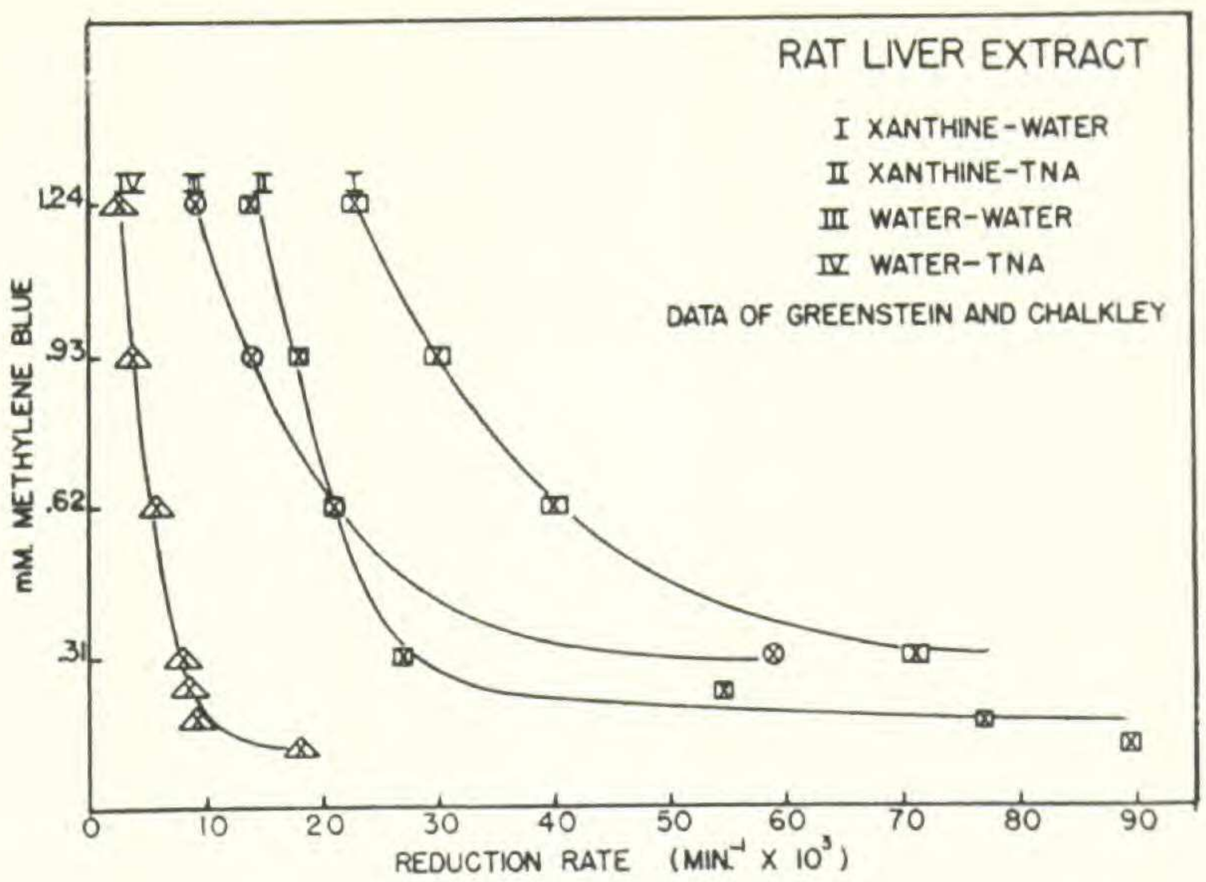

Figure 1. The relation between methylene blue concentration and the rate of decolorization of the dye. Ordinate refers to m M dye per cc. added to mixtures described by curve I consisting of 2 cc. extract + 1 cc. water + 1 cc. xanthine (1.6 m M); by curve II consisting of 2 cc. extract + 1 cc. 0.5 per cent sodium thymonucleate + 1 cc. xanthine (1.6 m M); by curve III consisting of 2 cc. extract + 2 cc. water; and by curve IV consisting of 2 cc. extract + 1 cc. water + 1 cc. 0.5 per cent sodium thymonucleate. Abscissa refers to rate of complete decolorization. Anaerobic conditions throughout. Temperature 24–26° C.

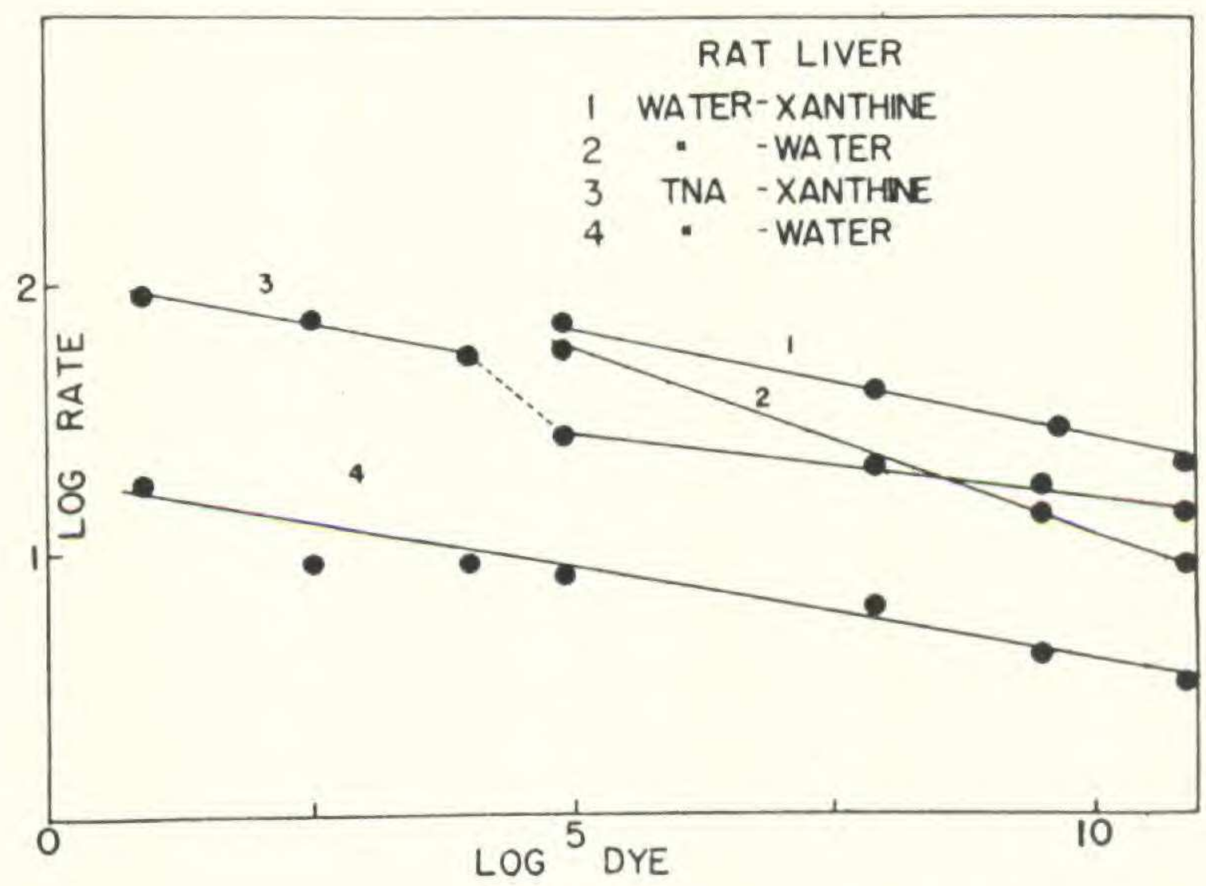

Figure 2. Relation between the logarithms of the methylene blue concentration and of the decolorization rates based upon data given in fig. 1. Composition of mixtures described by curves 1 and 4 are the same as that described by curves I and IV in fig. 1; that of curves 2 and 3 identical with that respectively of curves III and II in fig. 1.

TABLE I

EFFECT OF YEAST AND THYMUS SODIUM NUCLEATES ON THE DECOLORIZATION RATE OF METHYLENE BLUE IN RAT LIVER EXTRACTS*

Mixture†	Decolorization Rate
	Minutes $^{-1}$ x 10^3
1. 2 cc. liver extract + 1 cc. H_2O + 1 cc. H_2O + 1 cc. methylene blue	50
2. 2 cc. liver extract + 1 cc. H_2O + 1 cc. xanthine + 1 cc. methylene blue	83
3. 2 cc. liver extract + 1 cc. yeast nucleate + 1 cc. H_2O + 1 cc. methylene blue	40
4. 2 cc. liver extract + 1 cc. yeast nucleate + 1 cc. xanthine + 1 cc. methylene blue	71
5. 2 cc. liver extract + 1 cc. thymus nucleate + 1 cc. H_2O + 1 cc. methylene blue	7
6. 2 cc. liver extract + 1 cc. thymus nucleate + 1 cc. xanthine + 1 cc. methylene blue	38

* Concentration of methylene blue 1.24 x 10^{-3} M, of the nucleates 1.0%, of xanthine 1.6 x 10^{-8} M; each cc. of the aqueous liver extract equivalent to 300 mgms. of tissue; temperature 24–26° C. Anaerobic conditions throughout by employment of evacuated Thunberg tubes at approximately 18 mm. pressure.

† Xanthine dehydrogenase activity obtained by subtracting, respectively, 2. from 1., 4. from 3., and 6. from 5.

in terms of the logarithms of the respective variables with results described in fig. 2.

It is evident that there is a linear relation for all the mixtures at the higher dye concentrations, but there is a distinct break in curve 3 (fig. 2) at the lower dye concentrations. This break, symbolized by a dotted line since we do not know as yet its shape at this point, indicates an apparent increase in xanthine dehydrogenase activity. With progressively decreasing amounts of the methylene blue this increase in activity becomes progressively greater. The activity of xanthine dehydrogenase, although independent in the presence of desoxyribose nucleate at the higher levels of methylene blue concentration, is affected by the presence of the nucleate at the lower dye concentrations in such a way that it is apparently increased.

This apparent increase in dehydrogenase activity in the presence of the nucleate at low dye concentrations is all the more striking when the activity of this enzyme in the absence of added nucleate is considered. Inspection of curves 1 and 2 in fig. 1 suggests that these curves cross in the vicinity of the break in curve 3. The crossing of curves 1 and 2 means that with progressively lower dye concentrations the activity of xanthine dehydrogenase becomes apparently increasingly more negative. Further investigations have indeed revealed this to be the case. At very

low dye concentrations, the presence of the substrate, xanthine, interferes in some way with the ordinary processes of reduction of the dye by the tissue reducing systems. On the other hand, at these same low dye concentrations, when both substrate and nucleate are present, the rate of reduction of the dye is accelerated. Thus, with decreasing amounts of methylene blue, the divergence in the activity of the dehydrogenase in the presence of the nucleate or in its absence, is in opposite directions, i. e., the activity in the presence of nucleate becomes increasingly greater, the activity in the absence of nucleate becomes increasingly negative. The effect of the nucleate is thus markedly emphasized, and suggests qualitative as well as quantitative effects produced by this substance. No changes in pH are produced by the addition of the neutral sodium nucleates.

No explanation is apparent at the present time for the different directions which the activity of xanthine dehydrogenase takes in the presence and in the absence of desoxyribose nucleate. The results are quite reproducible from one extract to another. Further investigations on other enzyme systems and on other tissues are clearly desirable.[1]

Discussion

At this time any attempt at interpretation of the phenomena above described must of course be speculative. They are, from the biological viewpoint, the initial results of an attempted approach to the problem of the well-known role of the nucleus as co-ordinator of the functional activities of the cell. In this respect, it is perhaps permissible to note that, as far as the writers are aware, the results

[1] Since the above was written, more experiments involving a greater range of dye concentrations have been performed. These have shown that the relative rates of decolorization in the presence of nucleates may actually be accelerated when the dye concentration is sufficiently increased. It will be noted from fig. 2 above that curves 2 and 4 converge as the dye concentration is increased and presumably would cross at a relatively high dye concentration. The reality of such an intersection has now been experimentally established, and thus the existence of an accelerating effect of desoxyribose nucleate at sufficiently high dye concentrations has been proved. A similar study has also been made with respect to ribose nucleate, and it has been found that an analogous reversal of effect occurs, but at a substantially lower concentration of dye than that required for desoxyribose nucleate. Thus, at sufficiently low concentrations of dye the addition of either ribose nucleate or desoxyribose nucleate retards the decolorization rate; at sufficiently high concentrations of dye the addition of either nucleate accelerates the decolorization rate, whereas at intermediate concentrations of the dye the addition of ribose nucleate accelerates, and the addition of desoxyribose nucleate retards, the decolorization rate. The relative ranges of dye concentration where these three separate effects are noted will depend on the tissue used, the degree of dilution of the extract, and on the concentration of the nucleate.

Dilution of the extract may be taken as equivalent to reducing the amount of oxidizable substrate. Changes in dye concentration may be equated to normal hydrogen acceptor levels. Hence it would appear that for any given level of substrate and hydrogen acceptor the level of nucleates within the cell would determine the rate of dehydrogenation (anaerobic stage of oxidative metabolism). Since fluctuations in the release or production of nucleates within the cell may reasonably be assumed to be associated with gene action, we may be here dealing with the normal mechanism for control of general cellular metabolism—at least in so far as the initial stages of oxidative metabolism are concerned. The system or systems here studied are obviously highly complex and appear to exist in a delicately adjusted state of equilibrium. As a further example in addition to the phenomena listed above, the use of phosphate buffers normally considered innocuous, when added to tissue systems in such a way as not to disturb the pre-existing pH, markedly alters the quantitative aspects of the decolorization rates. Specific salt effects are thus also concerned in the kinetic mechanisms. Investigations are now in progress covering this and related aspects of the problem.

constitute the first direct chemical evidence that a typically nuclear constituent, desoxyribose nucleic (thymonucleic) acid can directly affect the enzymatic redox systems of the cell.

The fact that, in the presence of desoxyribose nucleate, the over-all activity of these systems is altered immediately suggests that differences in production or release of such nucleates within the cell could regulate its metabolic activities. It also appears, since the effect of the nucleate is apparently relatively, if not absolutely, reversed for xanthine dehydrogenase (the enzyme typically involved in purine metabolism) at low oxidative levels (i. e., low concentrations of methylene blue), that this regulative effect might extend to the metabolism of the nucleic acid itself. In that case the effect would apparently depend largely upon the concentration of the hydrogen acceptors available, up to and including oxygen.

One is tempted to recall the finding of Chalkley and Voegtlin ('40), that changes in oxygen tension strongly affect the growth and fission of the nucleus in *Amoeba proteus* and also the sulfhydryl cycle observed by Chalkley ('37) within the nucleus of the same organism, which last could conceivably produce a variation of redox equilibria within the nucleus, i. e., in the immediate vicinity of the desoxyribose nucleic acid, and which was shown to be correlated with the growth and fission of the nucleus. Considered in conjunction with the present data, these observations might lead to the suggestion that the oxygen supply together with desoxyribose nucleic acid metabolism might, through affecting the redox systems of the cell, constitute the regulating mechanism of the entire cell metabolism.

Further, the intimate relation of the desoxyribose nucleic acid to chromosome structure might suggest that gene activity expresses itself in no small part by means of this system.

As stated above, this is obviously speculation but at least there can be no doubt that we have at last definite physiologic, i. e., biochemical, action demonstrated to occur between a component limited to the nucleus and certain enzyme systems concerned in cell metabolism.

It is of interest to note that the ribose form of the acid (yeast nucleic acid) shows, if any, much less retardative activity. Thus the cytoplasmic form of the acid is sharply set off in this respect from the purely nuclear form.

The implications of these extremely simple experiments are far-reaching. It is obviously necessary to carry out greatly extended research, however, before any of the foregoing suggestions can have any merit other than that of serving as indications of our mode of approach.

SUMMARY

Aqueous extracts of rat and of mouse liver possess the capacity of reducing solutions of methylene blue. In the presence of desoxyribose nucleate the rate of decolorization of relatively low concentrations of dye is appreciably delayed, and the decrease in rate is proportional to the nucleate concentration.

At relatively high levels of dye concentration the activity of xanthine dehydrogenase in these extracts is the same whether desoxyribose nucleate is present or not. At low levels of the dye there is an apparent increase in the activity of the enzyme when desoxyribose nucleate is present.

Ribose or desoxyribose nucleate may accelerate or retard the reducing systems of the liver, the effect depending on the dye concentration.

The possible implications of these findings are discussed in the light of genic mechanisms.

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